1554-Pos Board B464
Altering the Length and Character of S3-S4 Loops Changes Equilibrium and Kinetic Properties of Kv1.2, as Revealed Through Mutagenesis and Molecular Dynamics Simulations
Rheanna Sand, Nazlee Sharmin, Warren J. Gallin.
Loops connecting core secondary structure elements of proteins are highly variable in length and sequence between homologs; as such they are often thought to have little or no participation in the function of many proteins. In the case of voltage-gated potassium channels, truncation of the loop connecting the S3 and S4 helices has been demonstrated to alter voltage sensitivity (Gonzalez et al. (2000) J. Gen. Physiol. 115: 193-208 and (2001) P.N.A.S. 98: 9617-23). We have studied the effect of replacing this loop with homopolymers of different length and composition, and compared these effects with results of molecular dynamics simulations of these short peptide sequences. After each simulation, a free energy profile was plotted as a function of end-to-end distance of the isolated loops, thus allowing a correlation between loop dynamics and voltage-sensitive opening and closing. Constraints on the distance between the C-terminus of the S3 helix and the N-terminus of the S4 helix affect both equilibrium and kinetic properties of mouse Kv1.2. The shortest loops, consisting of two amino acids, all lead to a strong positive shift in the V50 values for the channels, whereas long loops, where the end-to-end distance can exceed 1.5 nm, have smaller effects on the V50 while having significant effects on the kinetics of channel opening. Glutamate linkers cause significant kinetic slowing, but proline loops four residues in length show the most positive V50 and longest 10-90% rise time. Channels constructed with short loops of proline and serine also appear to conduct small amounts of inward current at a wide range of voltages prior to outward conductance. The G/V-derived Boltzmann slope factor was not significantly affected by altering the S3-S4 loop sequence.

1555-Pos Board B465
Analysis of the Contribution of Domain Swapping Interactions to *Shaker* Kv Channel Gating Reveals a Dynamic Upper Voltage-Sensor Pore Domain Interaction Surface
Tzilhav Shem-Ad, **Ofer Yifrach**.
Recent structural information on the Kv 1.2 potassium channel revealed two interaction surfaces between the voltage sensor and pore domains. While the lower interaction surface is intra-subunit and its contribution to the electromechanical coupling underlying channel opening is relatively understood, the upper (domain-swapped) interaction surface is inter-subunit and its contribution to the mechanism of Kv channel gating is not yet clear. Evolutionary information, mutagenesis and covalent cross linking analyses indicated that residues spanning the upper interface play an important role in Kv channel function. These analyses, however, did not provide mechanistic information regarding possible rearrangements associated with this interface during Kv channel gating. To further address the nature of the upper interaction surface, whether dynamic or static, we assessed the contribution of such inter-subunit domain swapping interactions to Kv channel gating by combining electrophysiology recordings of wild type and (upper interface) mutant proteins, introduced in the context of a tandem-dimer channel construct, with thermodynamic coupling analysis by means of double-mutant cycles formalism. Our results reveal that: (1) point mutations of pore and voltage-sensor residues at the upper interaction surface stabilize the closed channel state, (2) that pore-voltage sensor residue pairs across the upper inter-subunit interaction surface are coupled and (3) that the coupling is state-dependent and is stronger in the open channel state as compared to the closed state. Overall, our results suggest that the upper interaction surface is dynamic in nature and further support the assertion that the structure of the Kv channel solved is indeed that of the open channel state.

1556-Pos Board B466
R1 in the Shaker S4 Occupies the Gating Charge Transfer Center in the Resting State
Meng-chin A. Lin, Jui-Yi Hsieh, Allan F. Mock, **Diane M. Papazian**.
During Shaker activation, R1-R4 in S4 cross the transmembrane field. Recently, Tao et al proposed that F290 and E2 in S2 and D3 in S3 constitute a 'gating charge transfer center' occupied by R1 at rest (*Science* 328:67-73, 2010). In contrast, previous evidence suggests that R1 is extracellular to F290, near E1 and I287 in S2, at rest. We investigated the resting location of R1 using engineered Zn^{2+} binding sites in which I287H was paired with another histidine mutation. Importantly, binding sites involving I287H are located extracellular to the charge transfer center. In I287H+F324H, Zn^{2+} slowed opening, increased the delay before opening, and shifted the voltage dependence of the delay in the depolarized direction. These results indicate that Zn^{2+} binds to and stabilizes the resting state. In I287H+R1H, Zn^{2+} generated a slow component of activation. Its amplitude (A_{slow}) was ~55% in saturating Zn^{2+}, suggesting that only some voltage sensors can bind Zn^{2+} at −80 mV. The maximal A_{slow} decreased after depolarizing and hyperpolarizing prepulses. The decline of A_{slow} after negative prepulses supports the idea that R1 moves inward and occupies the charge transfer center upon hyperpolarization. Consistent with this, pairing I287H with A359H, located in the S3-S4 loop, generated a binding site. In high Zn^{2+}, a slow component of activation accounted for >80% of the kinetics, indicating that Zn^{2+} traps the voltage sensor in an absorbing conformation. We transferred I287H+A359H into the F290W+R1K+K5R background, which stabilizes the resting state. Zn^{2+} slowed activation kinetics, which were well fitted by one component in low Zn^{2+}. Therefore, stabilizing the resting state allows most I287H+A359H voltage sensors to bind Zn^{2+} at −80 mV. We conclude that R1 occupies the gating charge transfer center in the resting conformation. NIH-R01GM43459 (DMP)

1557-Pos Board B467
Molecular Determinants of Kv2.1 Channel U-Type Inactivation
John Azer, Christine Niven, Pouya Mafi, Charlene Allard, **Tom Claydon**.
Kv2.1 channels exhibit a U-shaped voltage-dependent inactivation that is thought to represent preferential inactivation from pre-open closed states. However, the molecular mechanism underlying Kv2.1 closed-state inactivation is not known. Here, we have performed a cysteine scan of the S3-S4 linker and S5-P-loop linker and discovered critical sites important in U-type inactivation of Kv2.1 channels. U-type inactivation was preserved in all S5-P-loop linker mutant channels, with the exception of E352C. The E352C mutation abolished U-type inactivation, whilst the E352Q mutation had no effect. Experiments with E352C treated with the reducing agent, DTT, re-established U-type inactivation as did the double mutant E352C/C241V. This suggests that a disulfide bond formed between residues 352C and C241 (in S2) prevents closed-state inactivation. The S3-S4 mutant, R289C, also reduced closed-state inactivation. In this case, application of MTSET restored WT-like U-type inactivation properties, suggesting importance of charge at this site. Kinetic modeling based on a previous scheme (Klemic et al. 1998 *Biophys J* 74;1779-89) suggests that both E352C and R289C mutant phenotypes resulted from a specific reduction in transitions into closed inactivated states. These data indicate that specific residues within the S3-S4 and S5-P-loop linkers play essential roles in closed-state inactivation of Kv2.1 channels.

Muscle Regulation II

1558-Pos Board B468
The Interplay of Chromatin and Transcription Factors in the Regulation of Muscle Differentiation
Wenjing Yang, Kambiz Mousavi, Hossein Zare, Vittorio Sartorelli, Weiqun Peng.
For eukaryotes, all genetic processes have to work with the DNA in the context of the chromatin structure. Chromatin modifications, such as histone modifications, play an essential role in gene regulation, cell development, and the origin of the diseases. Central to gene regulatory network is the binding of transcription factors to specific cis-regulatory elements in transcriptional regulation. How transcription factors interact with the local chromatin environment to determine affinity, specificity and functional output remains unclear.
In the regulatory process of skeletal myogenesis, master regulatory transcription factors, MyoD and MyoG, preside to the specification and differentiation of skeletal muscle cells from myoblasts into myotubes respectively. Using ChIP-Seq(Chromatin immunoprecipitation combined with high throughput sequencing) data for MyoD, MyoG in undifferentiated myoblast and differentiated myotubes skeletal muscle cells in C2C12 cell line, we found that the majority of MyoD and MyoG binding events occur at intergenic and intronic regions and are not immediately related to gene activation. What is more, we found that MyoD binding sites that disappeared after differentiation in general have weaker binding affinity than those that are retained, suggesting inhomogeneity of biophysical and regulatory functions. By combining the transcription factor binding data with genomic profiles of histone modifications, we found that for the MyoD

binding sites retained in the differentiation process, histone modifications H3K4m1 and H3K4me3 exhibit a dip centered at the binding sites and sandwiched by two peaks; Whereas for MyoD binding sites that disappear after differentiation, histone modifications H3K4m1 and H3K4me3 exhibit a single peak centered at the binding sites. Other histone modifications also exhibit different behaviors at the two groups of MyoD binding sites. We will discuss the possible causes of this drastically different behavior and their functional implications.

1559-Pos Board B469

Foxo1 Nuclear-Cytoplasmic Movement in Live Skeletal Muscle Fiber

Tova Neustadt Schachter, Martin F. Schneider.

The transcription factor Foxo1 is integral to the regulation of expression of proteins which promote muscle atrophy. Phosphorylation of Foxo1 causes its translocation to the cytoplasm and thus prevents Foxo1-DNA binding and consequent transcription of genes that cause muscle atrophy and cell death. Thus, phosphorylation of Foxo1 leads to cell survival and muscle hypertrophy. Maintenance of Foxo1 phosphorylation and its resulting cytoplasmic retention could be used to suppress muscle atrophy and thereby shift the atrophy/hypertrophy balance in favor of hypertrophy. This in turn may be utilized to develop therapeutic avenues in treatment of muscle wasting as seen in patients with denervation, age-related muscle wasting, and AIDS. Akt and serum- and glucocorticoid-inducible kinase (SGK) are important regulators of the phosphorylation status of Foxo1. These pathways have been well characterized and the effects on Foxo1 localization have been reported. However, the mechanisms which regulate nuclear influx and nuclear efflux have not been separately evaluated. Here, we determine the effects of Akt kinase activity specifically on nuclear influx of Foxo1. To accomplish this goal, we quantified nuclear and cytoplasmic levels of adenovirally expressed Foxo1-GFP in cultured flexor digitorum brevis. The nuclear influx during treatment with kinase inhibitor Akt IV alone and in combination with the nuclear efflux inhibitor lemptomycin B provides insight into the activity of Akt as a Foxo1 kinase. Surprisingly, Akt inhibition reveals Akt to have little effect on the rate of nuclear influx of Foxo1. To evaluate translocation of endogenous Foxo1 in a similar manner immunocytochemistry and western blotting techniques were used. These results indicate that Akt either phosphorylates Foxo1 primarily in the nucleus and not in the cytoplasm, or that there is a mechanism for cytoplasmic phosphorylation of Foxo1 other than via Akt. Supported by NIH-NIAMS Grants R01-AR056477 and T32-AR007592.

1560-Pos Board B470

Dynamic Skeletal Muscle Exercise and Fatigue Development in Post Infarction Heart Failure Rats and Effect of Warm-Up

Kristin H. Hortemo, Ole M. Sejersted, **Per K. Lunde**.

Skeletal muscle fatigue develops during physical exercise. Most reports on skeletal muscle fatigue use isometricfatigue protocols with a fall in force production as a measure of fatigue. However, most muscles shorten during normal use and we propose that the fatigue mechanism will be different during dynamic contractions as compared with isometric contractions.

Methods: Fatigue was induced in rat soleus muscles from post infarction heart failure (HF) rats and controls (SHAM) by in situ isotonic shortening contractions (1s at 30Hz every 2s for 15min). The muscles were allowed to shorten isotonically against a load (AL) corresponding to 1/3 of maximal isometric force. After 15min recovery period the muscles were stimulated with a similar protocol. MLC2s phosphorylation and metabolites were measured at different points of time.

Results: During 1st protocol, fatigue development was confirmed by a significant fall in shortening in both SHAM and HF. After 100s of stimulation soleus from SHAM showed an increase in baseline tension (Tbl) between stimulation trains, which was much less pronounced in HF ($p<0.01$). At this time there was a significant higher lactate concentration in SHAM muscle compared with HF. The increase in Tbl at 100s was correlated to both isotonic and isometric relaxation rate. At 100s in 2nd protocol, the isotonic and isometric relaxation rates were nearly recovered leading to almost no rise in Tbl in both SHAM and HF. There was a linear correlation ($p=0.99$) between maximal shortening and MLC2s phosphorylation in protocols with variable AL. We propose that MLC2s phosphorylation participates in the regulation of shortening, while metabolites (especially lactate) may contribute to the reduced relaxation rates leading to increased Tbl.

1561-Pos Board B471

Time Resolved Analyses of Gene Expression in a Rodent Icu Model

Monica Llano-Diez, Ann-Marie Gustafson, Hanna Goransson, Carl Olsson, Lars Larsson.

Intensive care unit (ICU) patients commonly develop severe skeletal muscle wasting that aggravate the recovery from the primary disease and weaning from respirator. The modern treatment in anesthesiology and intensive care can progress in Acute Quadriplegic Myopathy (AQM). This study aims at improving our understanding of the mechanisms underlying the muscle wasting and weakness in ICU patients with AQM. Specific interest is focused on duration-dependent effects on intracellular signaling and myofibrillar gene and protein expression. For that reason, a unique experimental rat model mimicking ICU such as mechanical ventilation, muscle unloading, neuromuscular blocking agents (NMBA) administration and monitoring at different time points from 6h to 14 days, was used. Gene expression profile was analyzed in gastrocnemius muscle, showing an increased expression in the muscle-specific ubiquitin ligases, atrogin-1 and MuRF-1 after 6 h, as well as other genes involved in translational repression, authophagic/lysosomal genes (LC3b, cathepsins), oxidative stress response and up-regulation of pro-apoptosis signaling, except caspase-3 that increased after 9 days of intervention. Metalloproteins, GST, cystatins and cell cycle repressors were up-regulated in the early stages in response to oxidative stress and cellular damage among other genes, while LIM and sarcomeric proteins, collagen, extracellular matrix transcripts, carbohydrate metabolism, mitochondrial genes and the muscle-specific calpain-3 were down-regulated mainly after 5 days.

These results suggest a very complex, unique and highly temporally coordinated activation of protein synthesis, degradation, protective mechanisms and intracellular signaling activation at different time points during ICU conditions.

1562-Pos Board B472

A novel Splicing Factor that Affects Titin Alternative Splicing

Wei Guo, Marion Greaser, Shijun Li, Herbert Schulz, Kathrin Saar, Michael Radke, Timothy A. Hacker, Kurt W. Saupe, Padmanabhan Vakeel, Thirupugal Govindarajan, Norbert Hubner, Michael Gotthardt.

Cardiac muscle expresses predominantly larger N2BA titin isoforms at embryonic and prenatal stages of development, and these are mostly replaced with a smaller N2B isoform in adults. We have previously discovered a mutation in rats that dramatically alters titin splicing (Greaser et al J Mol Cell Cardiol 44:982, 2008). To determine the mechanism responsible for this change in titin splicing, we performed genetic linkage analysis with 191 animals from two different backcrosses. The titin splicing factor mutation was mapped to the long arm of chromosome 1 using a 10K SNP chip. PCR verified that the mutation occurs as a large deletion of an RS type splicing factor. The deletion was further verified by Southern blot, qPCR, and western blot analysis. Immunofluorescence staining of cardiomyocytes and HL1 cells indicated that the splicing factor was localized in the nucleus. Insertion of adenovirus constructs of the factor into homozygous mutant cardiomyocytes restored wild type titin splicing. The mutant rats show significantly larger left ventricle (LV) diameter in diastole and lower ejection fractions. Heart rate response to dobutamine was blunted in both heterozygote and homozygote mutants compared to wild type. Histological observations after Masson trichrome staining showed that fibrosis was significantly increased in LV from the same groups as compared to wild type hearts. An increased percentage in sudden death occurred in heterozygotes and homozygous mutants after 10 months of age. The splicing factor is mainly expressed in cardiac muscle and skeletal muscle as determined by Western blotting. Although heterozygote and homozygote mutants survive and reproduce, the titin splicing factor is required for normal cardiac structure and function. Supported by NIH HL77196.

1563-Pos Board B473

Preventing Ryanodine Receptor 1 calcium Leak Improves Age-Dependent Muscle Dysfunction

Daniel C. Andersson, Mathew J. Betzenhauser, Steven Reiken, Albano C. Meli, Wenjun Xie, Alain Lacampagne, Andrew R. Marks.

A hallmark of mammalian aging is the progressive decline in muscle function, referred to as sarcopenia. It is commonly found that the force-generating capacity of aged muscle is reduced when normalized to the muscle cross-